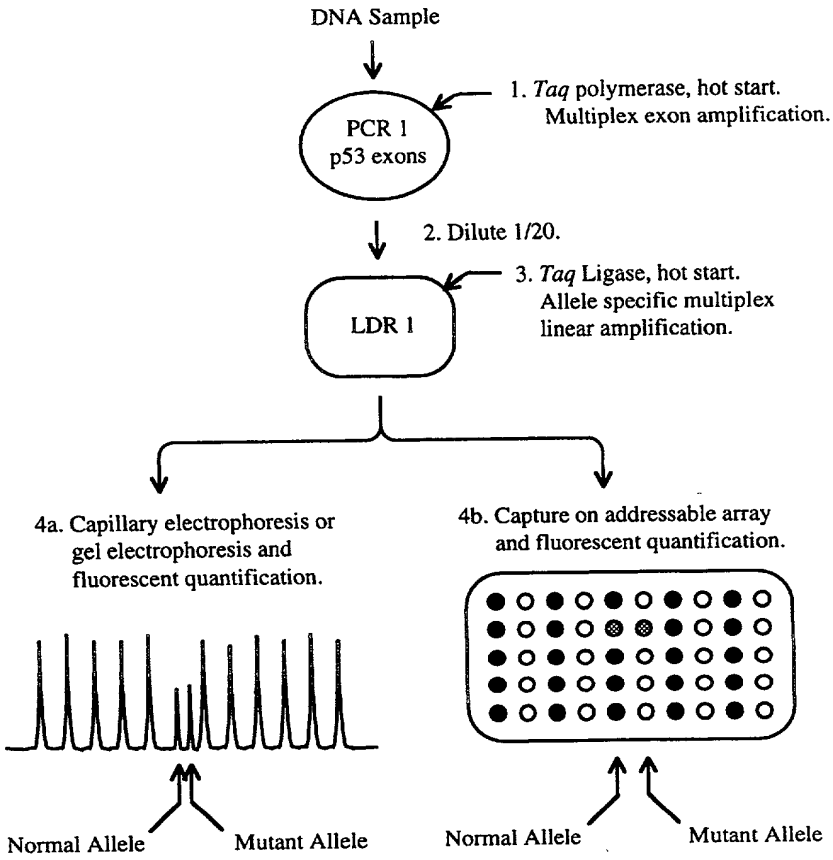

DNA Sample
1. Taq polymerase, hot start.
Multiplex exon amplification.
PCR 1
p53 exons
2. Dilute 1/20.
3. Taq Ligase, hot start.
Allele specific multiplex
linear amplification.
LDR 1
4a. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
4b. Capture on addressable array
and fluorescent quantification.
Normal Allele
Mutant Allele
Normal Allele
Mutant Allele

FIG. 1

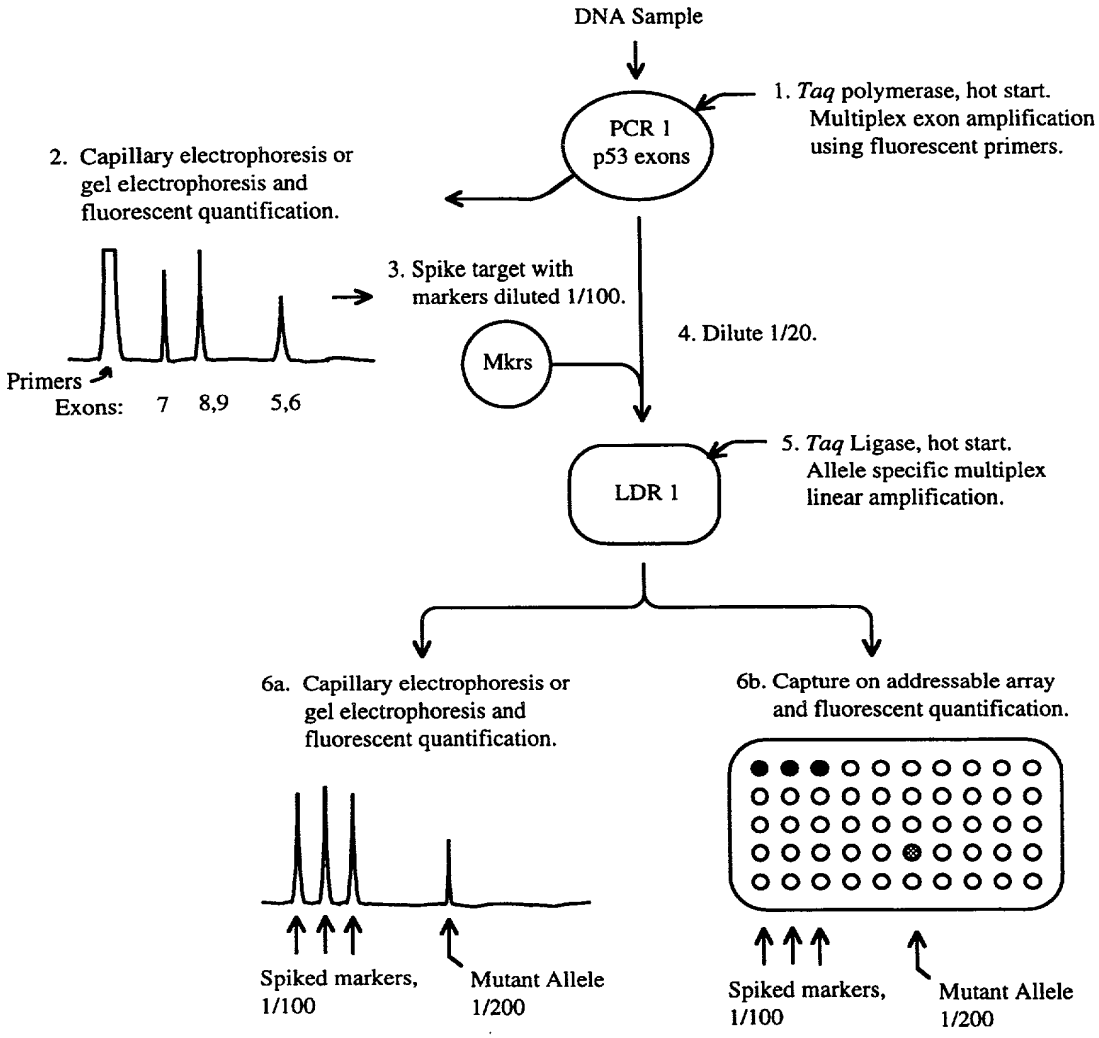
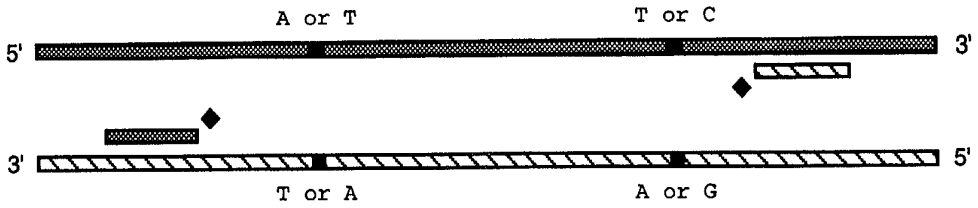

DNA Sample
1. *Taq* polymerase, hot start. Multiplex exon amplification using fluorescent primers.
PCR 1 p53 exons
2. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
3. Spike target with markers diluted 1/100.
Mkrs
4. Dilute 1/20.
Primers
Exons: 7 8,9 5,6
LDR 1
5. *Taq* Ligase, hot start. Allele specific multiplex linear amplification.
6a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
6b. Capture on addressable array and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200
Spiked markers, 1/100
Mutant Allele 1/200

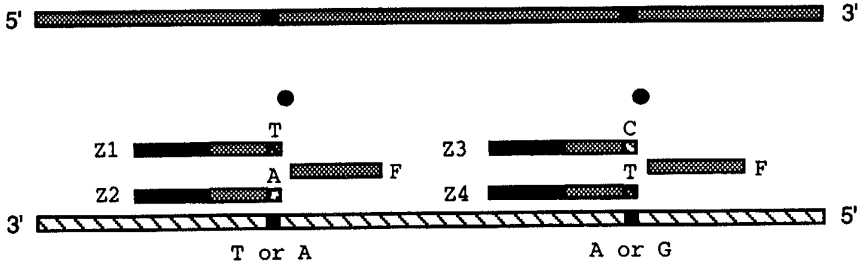
FIG. 2

PCR/ LDR

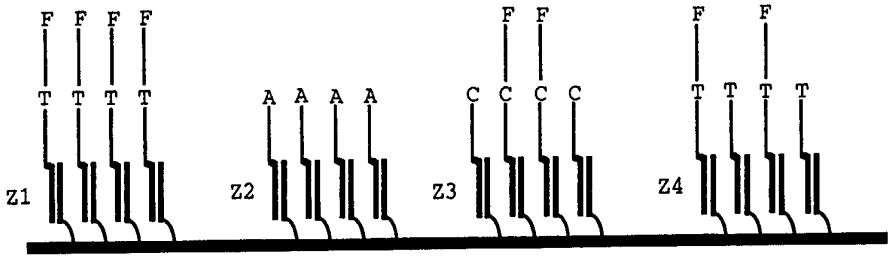
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

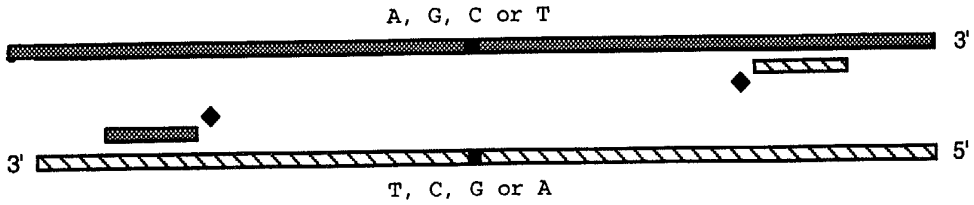


Homozygous: T allele only. Heterozygous: C and T alleles.

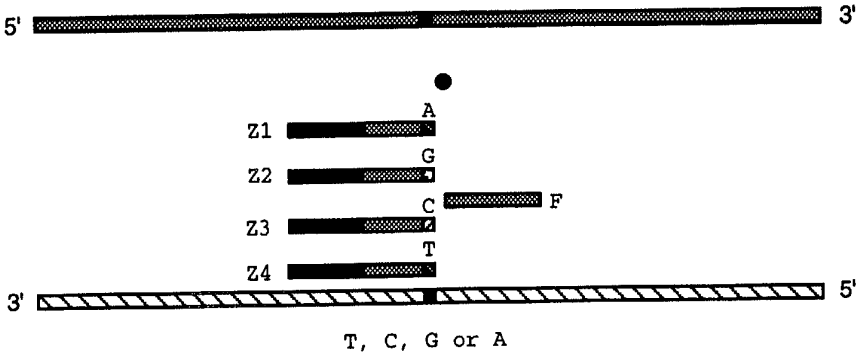
FIG. 3

PCR/ LDR

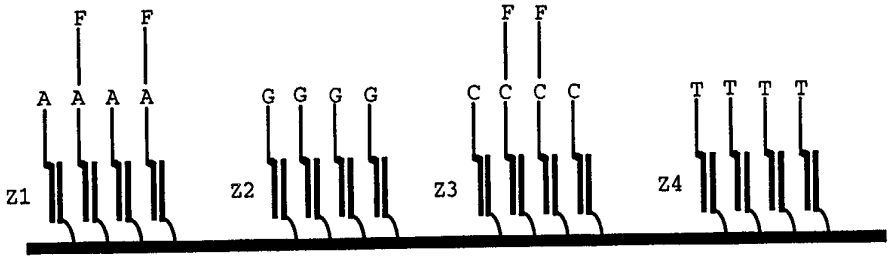
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

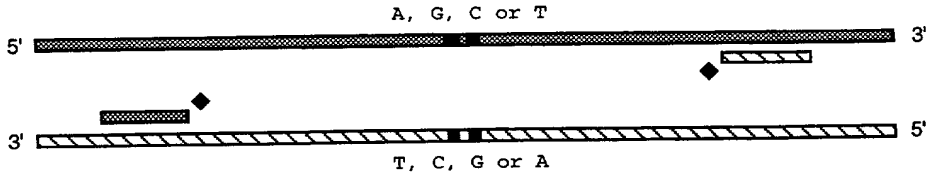


Heterozygous: A and C alleles.

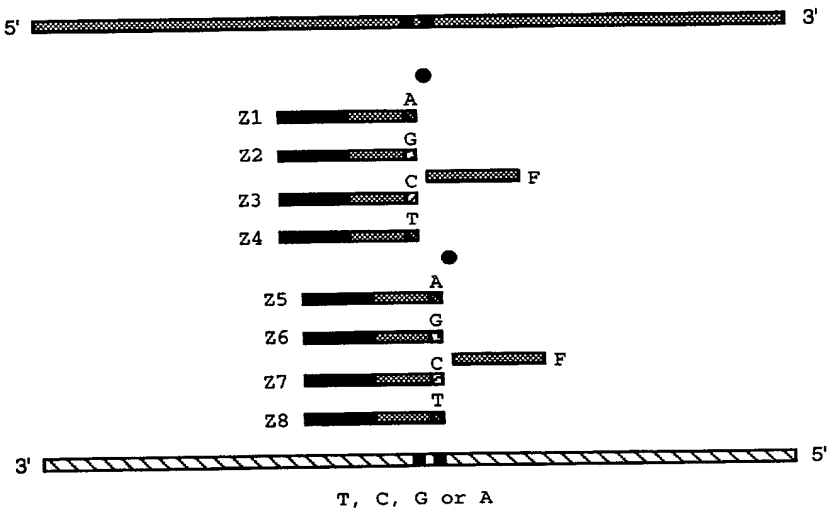
FIG. 4

PCR/ LDR : Nearby alleles

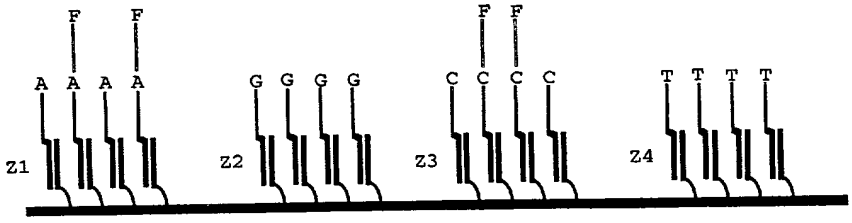
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



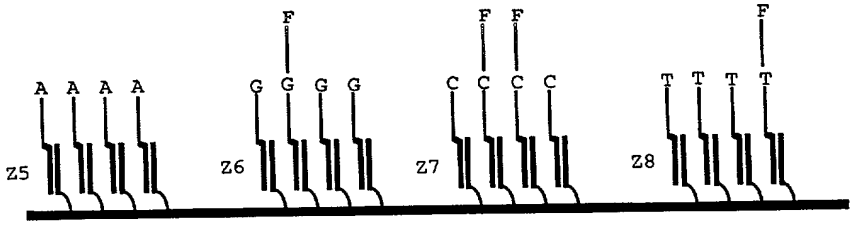
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A and C alleles.

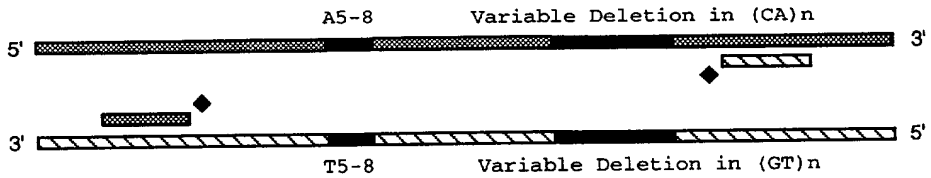


Heterozygous: G,C, and T alleles.

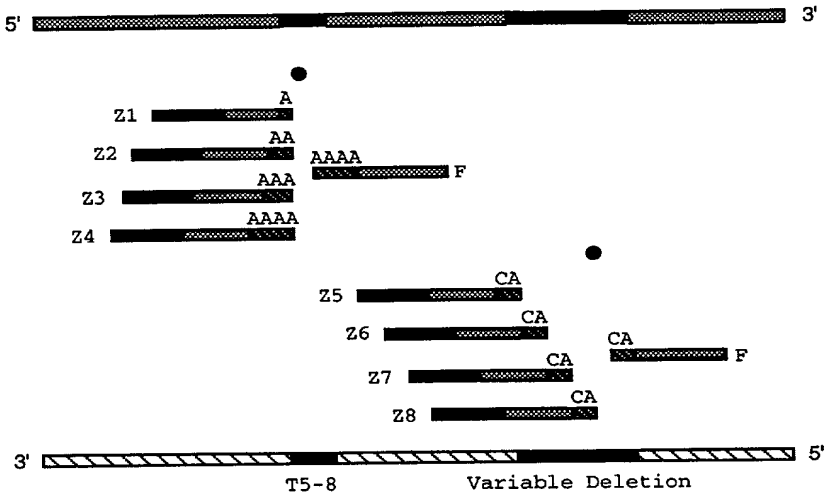
FIG. 5

PCR/ LDR : Insertions and Deletions

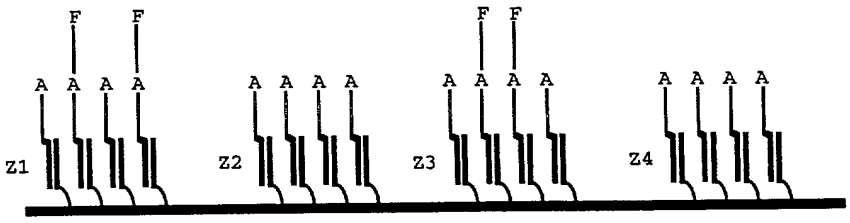
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



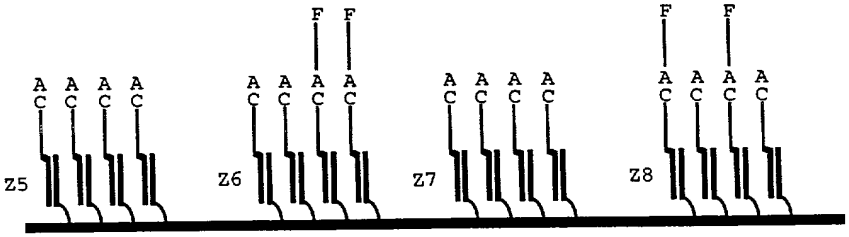
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: A5 and A7 alleles.

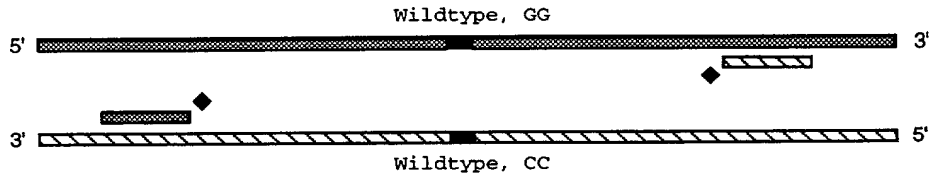


Heterozygous: (CA)5 and (CA)3 alleles.

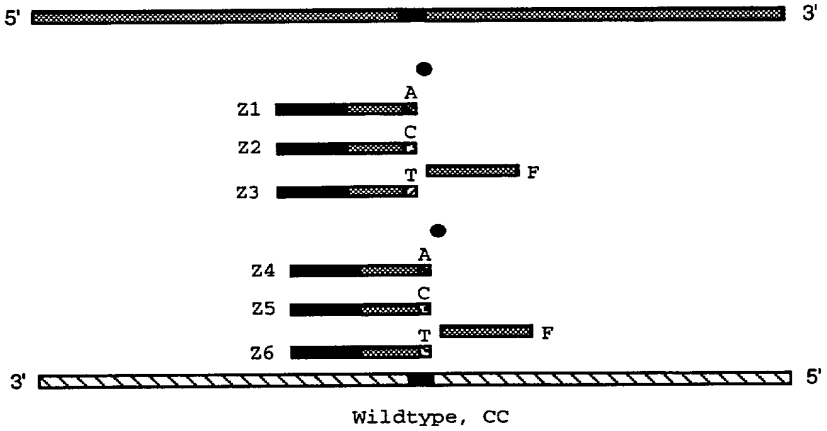
FIG. 6

PCR/ LDR : Adjacent alleles, cancer detection

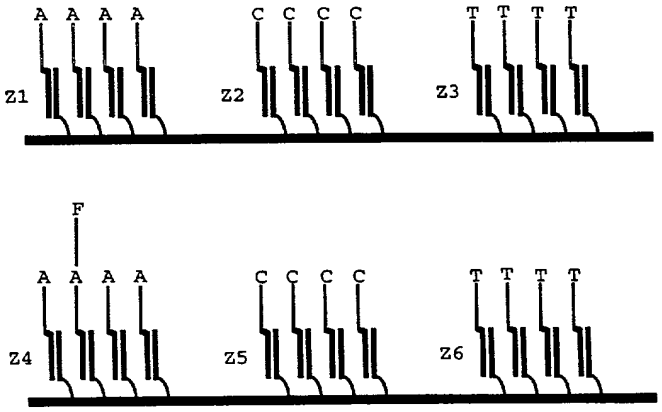
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

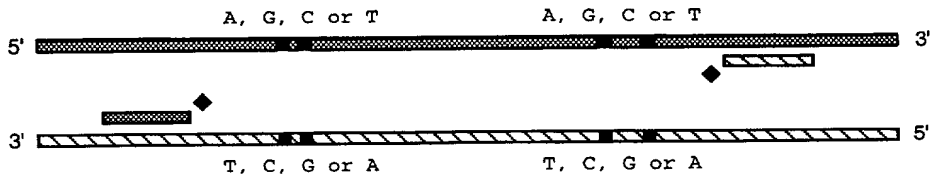


Gly to Asp mutation

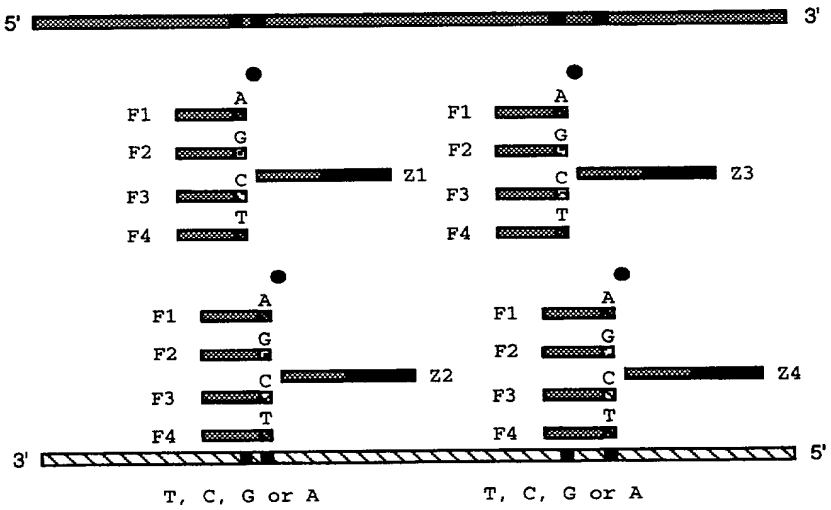
FIG. 7

PCR/ LDR : Nearby alleles

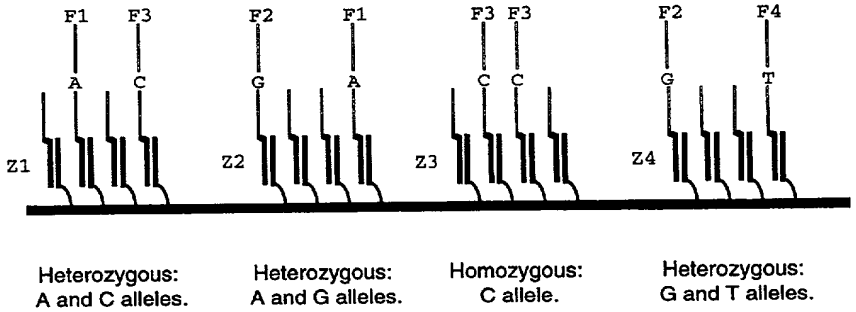
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.

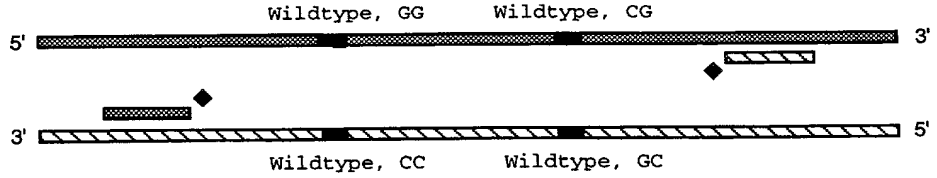


Heterozygous: A and C alleles. | Heterozygous: A and G alleles. | Homozygous: C allele. | Heterozygous: G and T alleles.

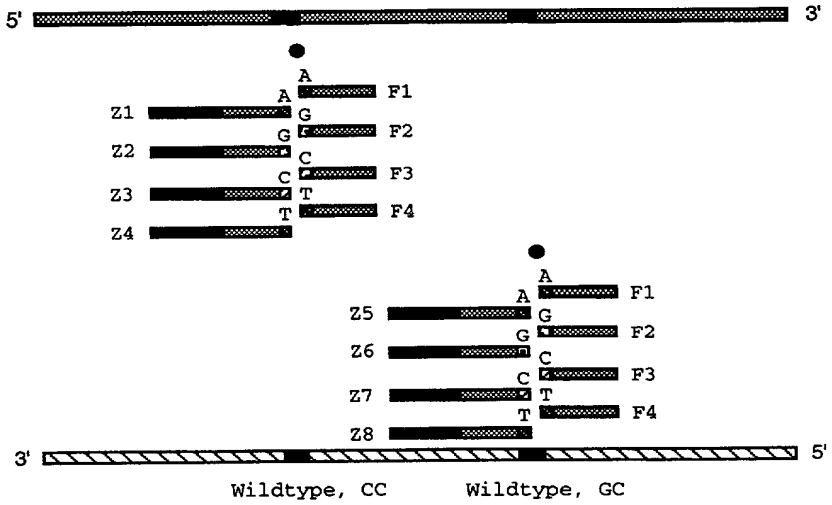
FIG. 8

PCR/ LDR : Adjacent and Nearby alleles

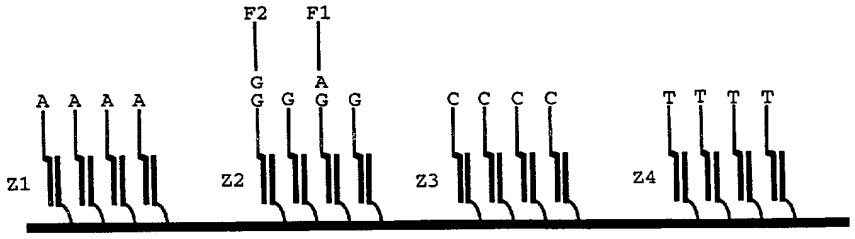
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



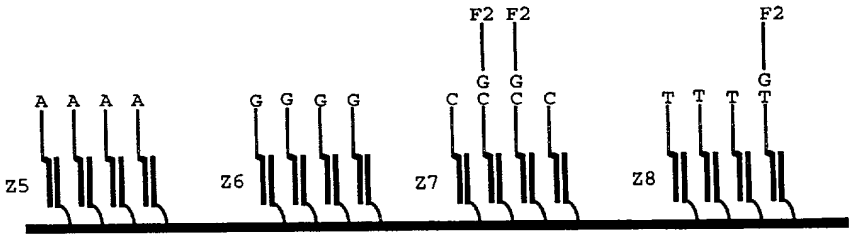
2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: Gly and Glu alleles.

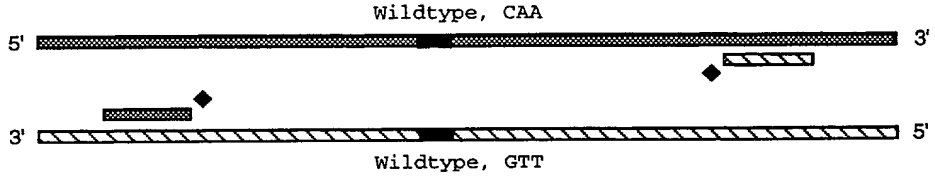


Heterozygous: Arg and Trp alleles.

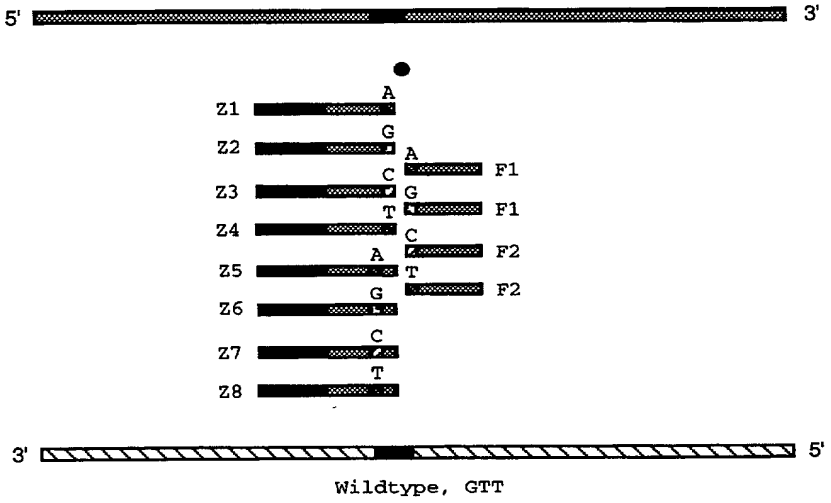
FIG. 9

PCR/ LDR : All alleles of a single codon

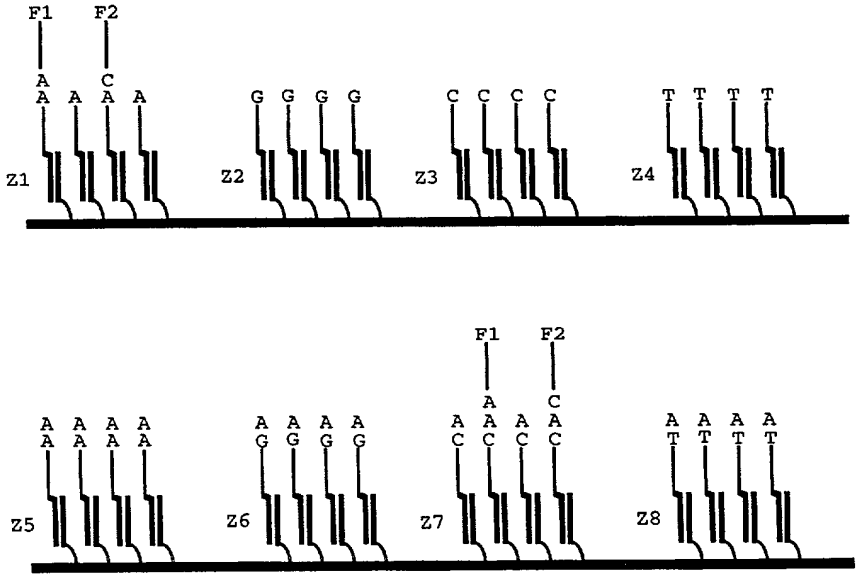
1. PCR amplify region(s) containing mutations using primers, dNTPs and *Taq* polymerase.◆



2. Perform LDR using allele-specific LDR primers and thermostable ligase.● Allele specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

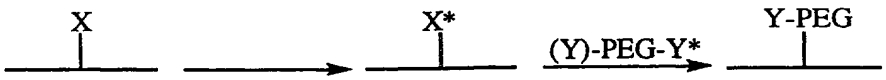


3. Capture fluorescent products on addressable array and quantify each allele.



Heterozygous: Gln and His alleles.

FIG. 10



X,Y = –OH, –CO_2H, –NH_2
(Y) = W–NH–

X*, Y* = –O(C=O)Z, –O(C=S)Z, –CO_2H, –(C=O)Z, –NH_2, –N=C=O

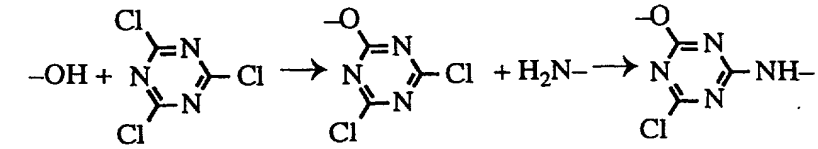
W = protecting group, e.g. Boc, Fmoc
Z = activating group, e.g. imidazole (Im), *p*-nitrophenol (OPnp), hydroxysuccinimide (OSu), pentafluorophenol (OPfp)
PEG = oligo or poly(ethylene glycol), backbone $(CH_2CH_2O)_n$ n = 6 to 200 (can also be grown by anionic polymerization with )
WSC = water soluble carbodiimide

Functional group transformations/activation (as needed), X → X*, Y → Y*

–OH ⟶ $–O(CH_2)_nCO_2H$ n = 1, 2
–OH ⟶ $–O(C=O)NHCH_2CO_2H$
–OH ⟶ $–O(C=O)CH_2NH_2$
–OH ⟶ –O(C=O)Im
–OH ⟶ $–O(C=S)SCH_2(C=O)NH_2$
$–CO_2H$ ⟶ $–(C=O)NH(CH_2)_nNH_2$ n = 2,6
$–CO_2H$ ⟶ –(C=O)Z
$–NH_2$ ⟶ $–NH(C=O)(CH_2)_nCO_2H$ n = 2, 3

Covalent linkage, X* + Y*

$–CO_2H + H_2N– + WSC + HOSu$ ⟶ –(C=O)NH–
–OH + Im(C=O)Im + $H_2N–$ ⟶ –O(C=O)NH–



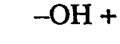
–OH + O=C=N– ⟶ –O(C=O)NH–
$–O(C=S)SCH_2(C=O)NH_2 + H_2N–$ ⟶ –O(C=S)NH–
–OH +  + HO– ⟶ $–OCH_2CH(OH)CH_2O–$
(+ $H_2NH–$) (NH–)
–OH ⟶ $–OCH_2(C=O)H + H_2N– + NaCNBH_3$ ⟶ $–OCH_2CH_2NH–$

FIG. 11

FIG. 12A

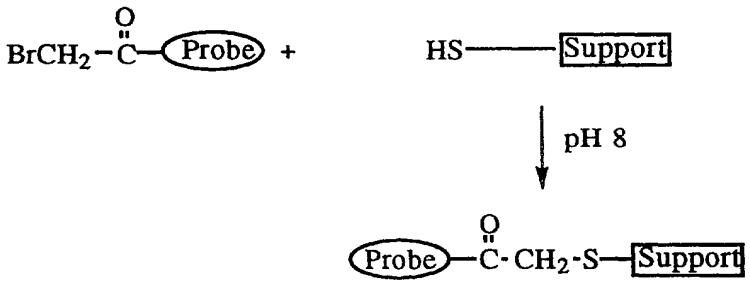


FIG. 12B

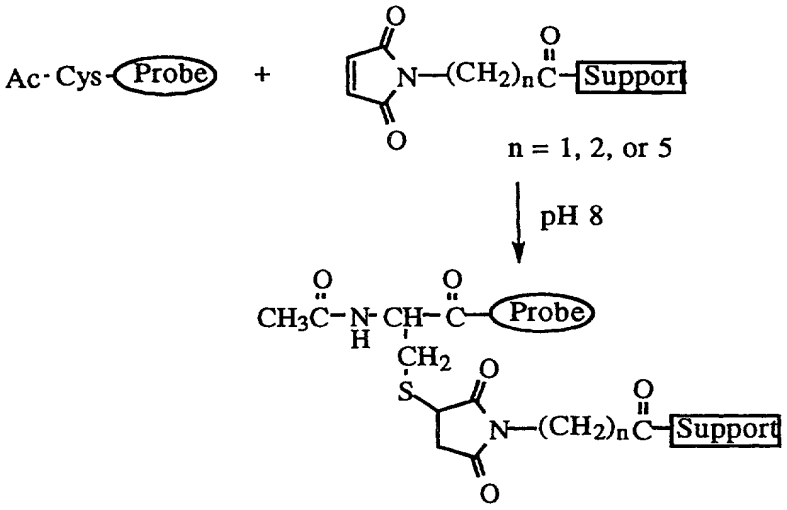
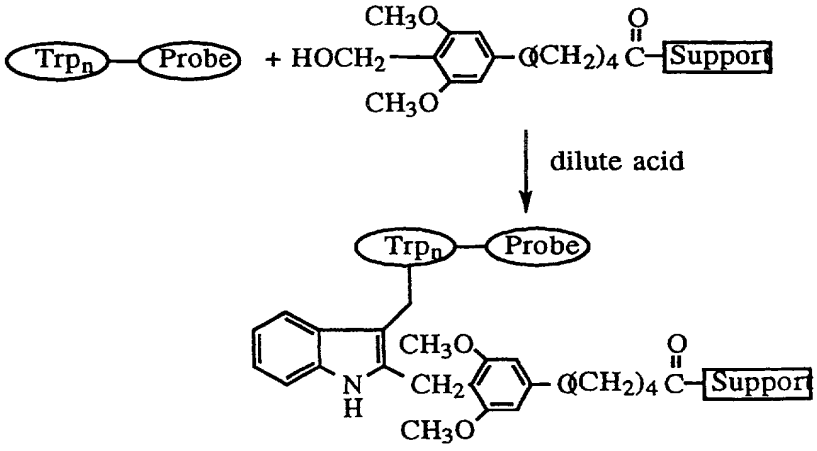


FIG. 12C



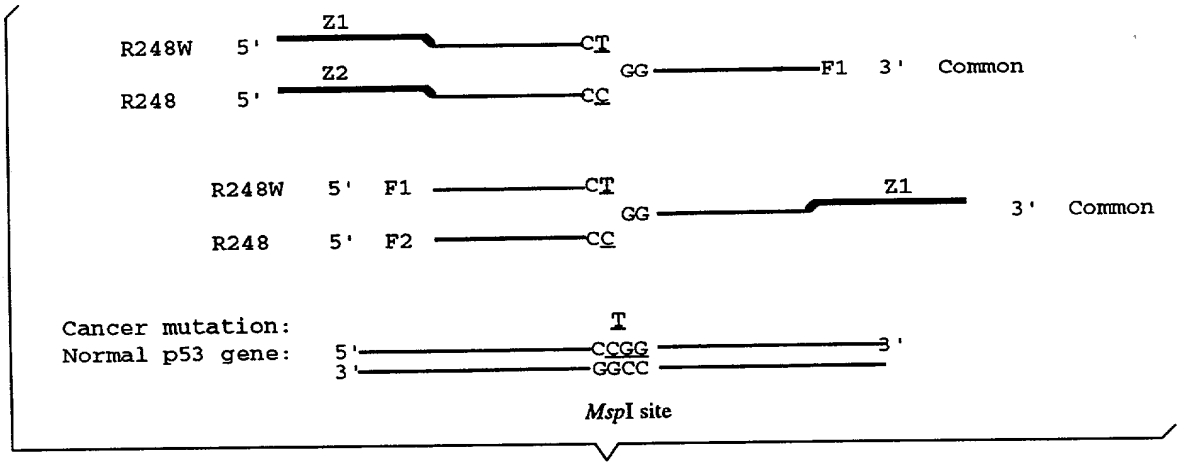


FIG. 13A

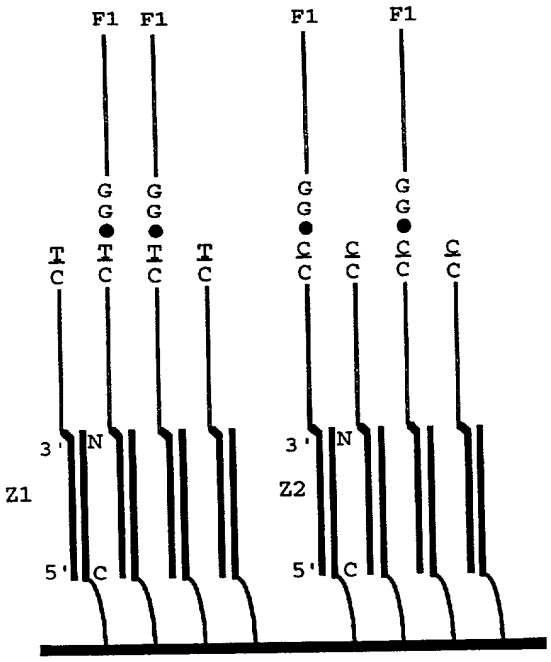


FIG. 13B

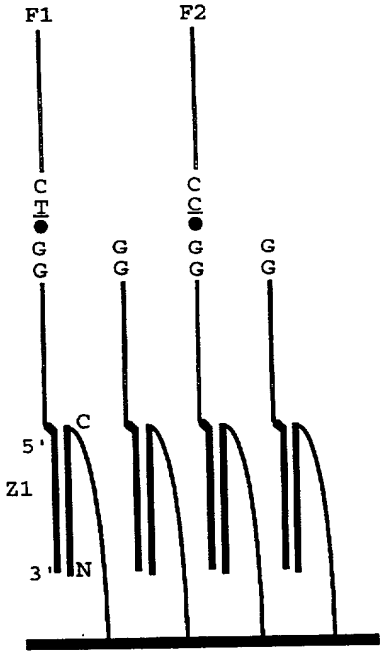


FIG. 13C

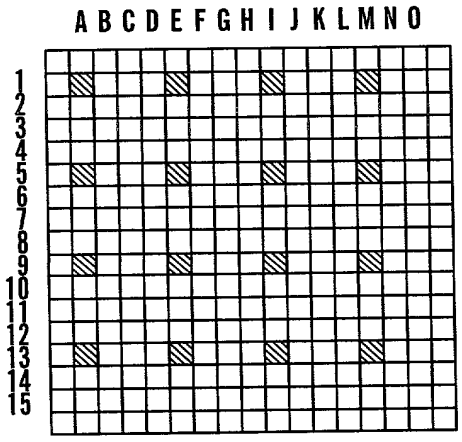


FIG. 14A

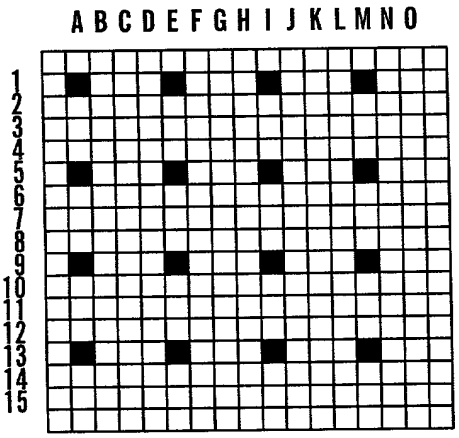


FIG. 14B

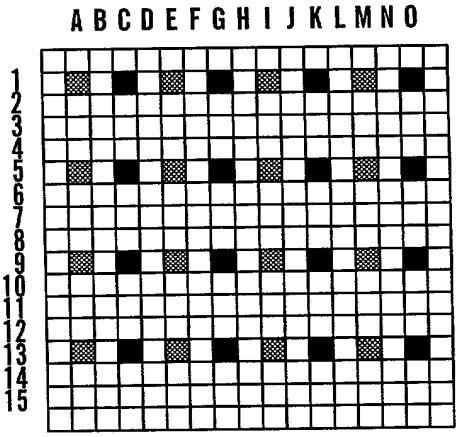


FIG. 14C

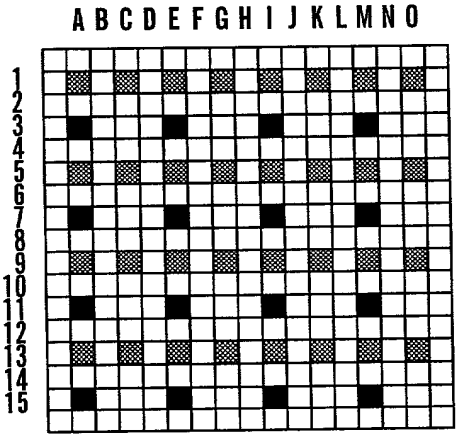


FIG. 14D

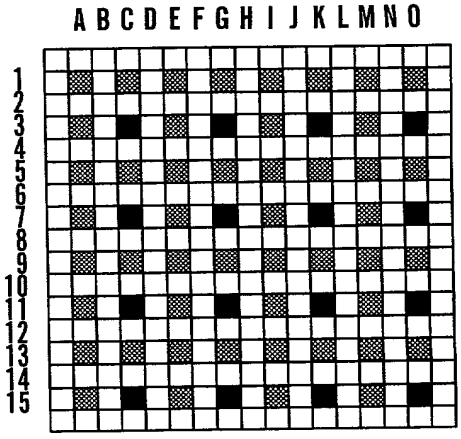


FIG. 14E

FIG. 15A

1st addition of unique 24mers.

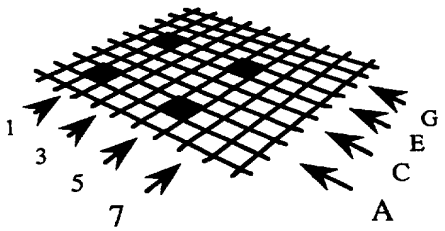


FIG. 15B

2nd addition of unique 24mers.

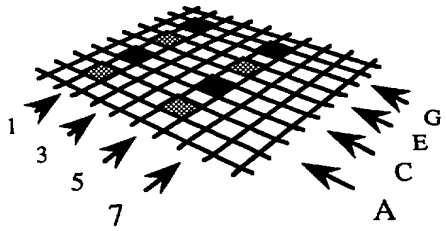


FIG. 15C

3rd addition of unique 24mers.

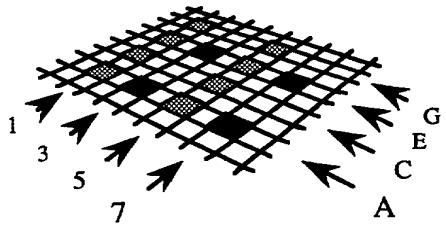
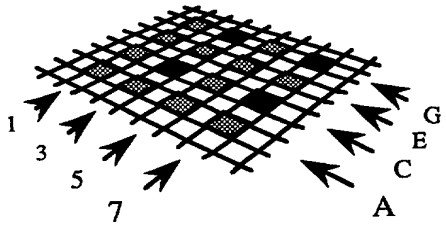


FIG. 15D

4th addition of unique 24mers.



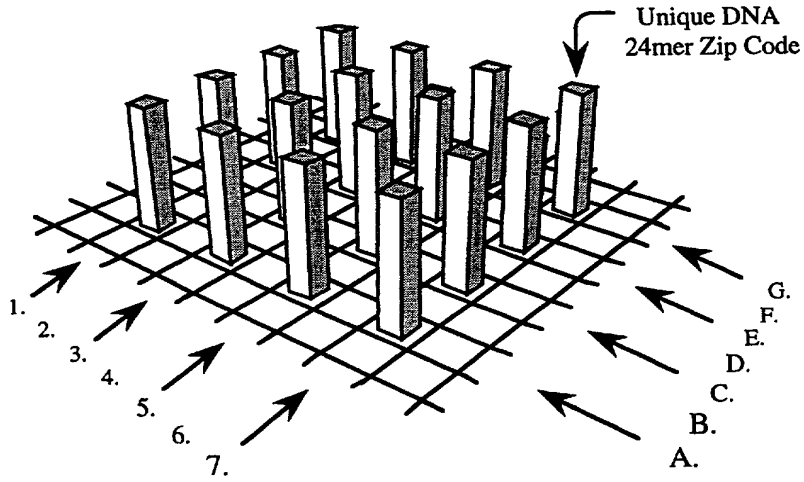


FIG. 15E

FIG. 16A

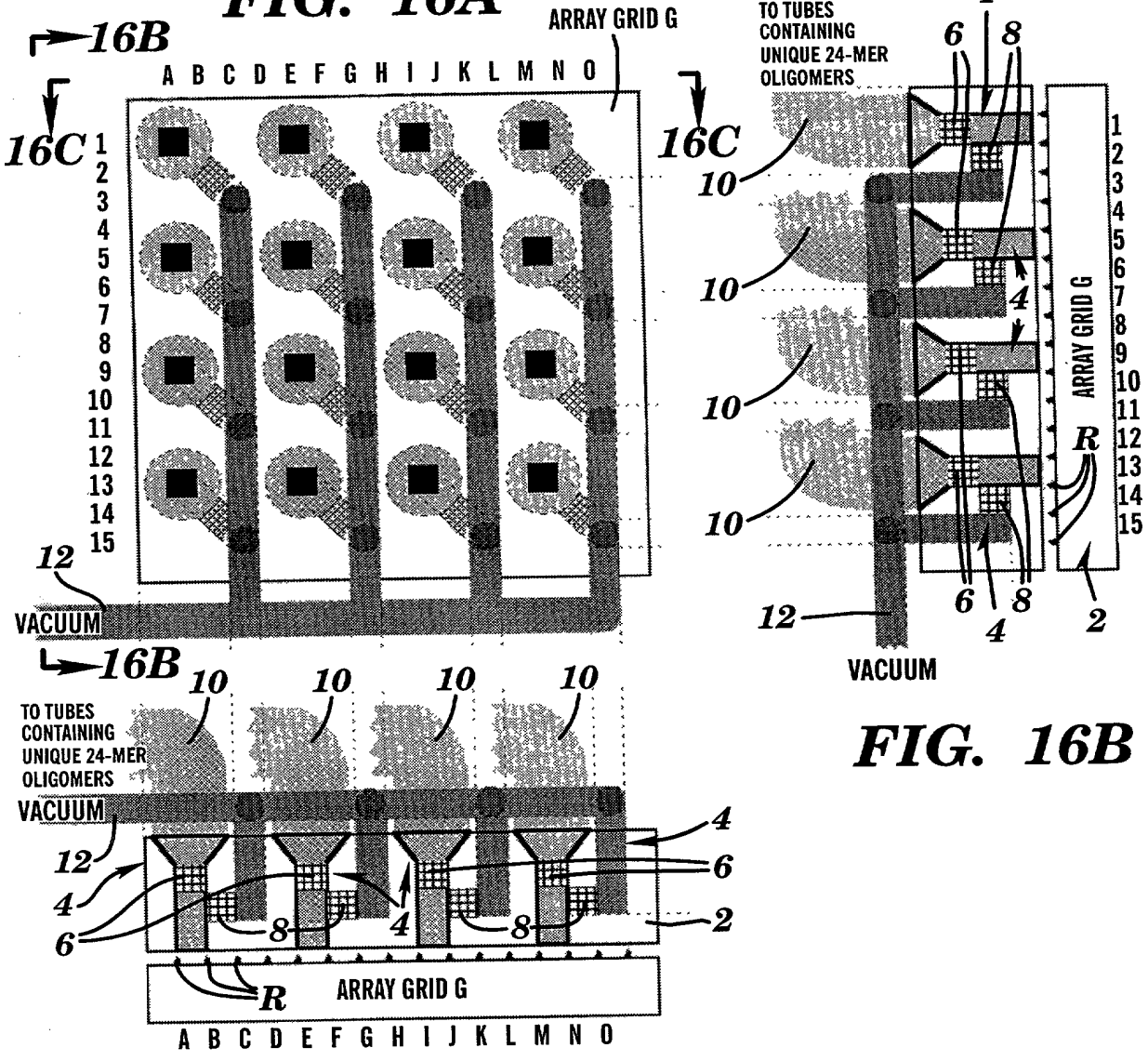


FIG. 16B

FIG. 16C

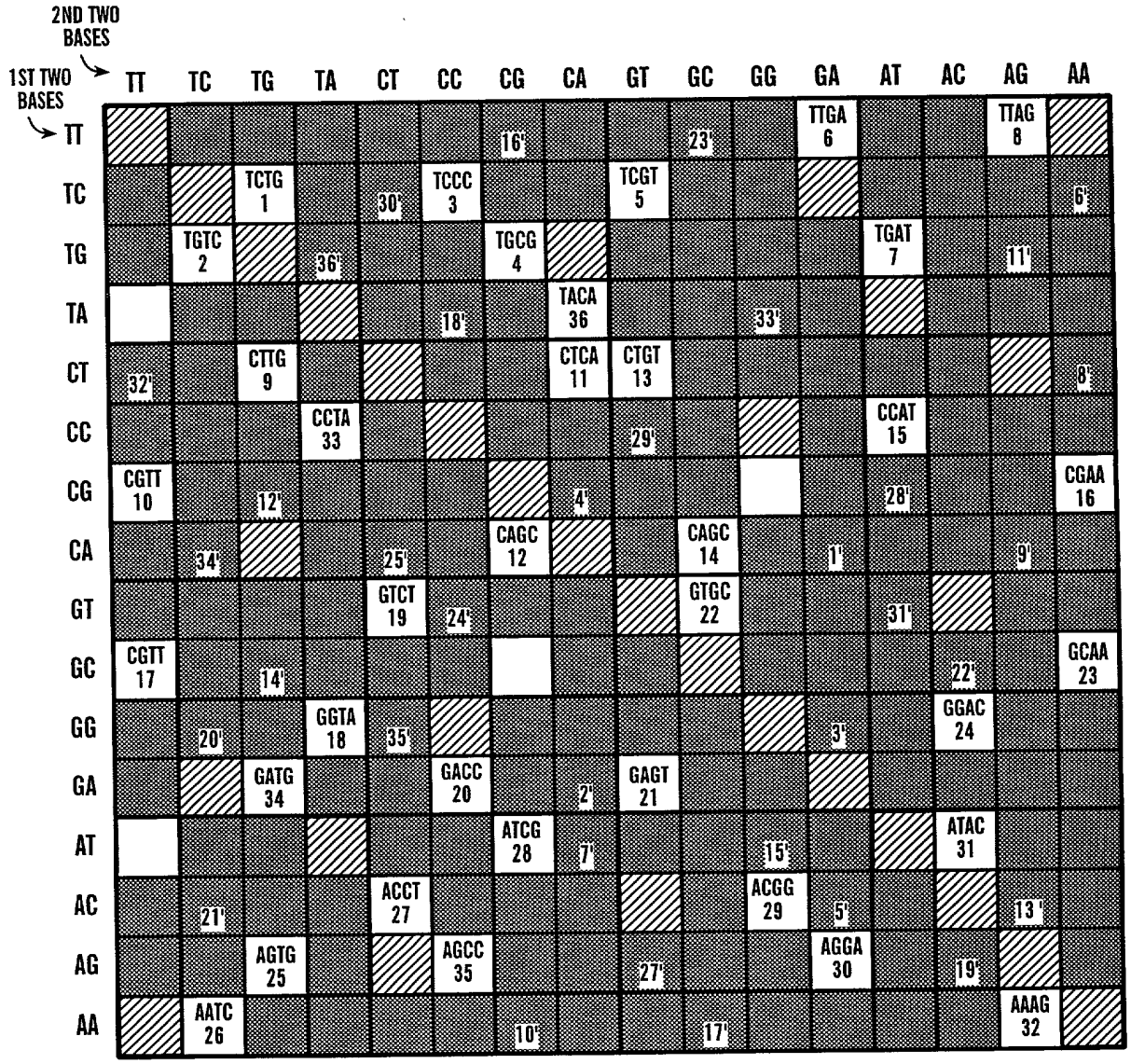


FIG. 17

1st Tetramer addition
(columns)

1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5

FIG. 18A

2nd Tetramer addition
(rows)

6	6	6	6	6
5	5	5	5	5
4	4	4	4	4
3	3	3	3	3
2	2	2	2	2

FIG. 18B

3rd Tetramer addition
(columns)

3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1
3	4	5	6	1

FIG. 18C

4th Tetramer addition
(rows)

2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5
4	4	4	4	4

FIG. 18D

5th Tetramer addition
(columns)

6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4
6	1	2	3	4

FIG. 18E

6th Tetramer addition
(rows)

3	3	3	3	3
2	2	2	2	2
1	1	1	1	1
6	6	6	6	6
5	5	5	5	5

FIG. 18F

Addressable array with full length PNA 24mers

1-6-3-2-6-3	2-6-4-2-1-3	3-6-5-2-2-3	4-6-6-2-3-3	5-6-1-2-4-3
1-5-3-1-6-2	2-5-4-1-1-2	3-5-5-1-2-2	4-5-6-1-3-2	5-5-1-1-4-2
1-4-3-6-6-1	2-4-4-6-1-1	3-4-5-6-2-1	4-4-6-6-3-1	5-4-1-6-4-1
1-3-3-5-6-6	2-3-4-5-1-6	3-3-5-5-2-6	4-3-6-5-3-6	5-3-1-5-4-6
1-2-3-4-6-5	2-2-4-4-1-5	3-2-5-4-2-5	4-2-6-4-3-5	5-2-1-4-4-5

FIG. 18G

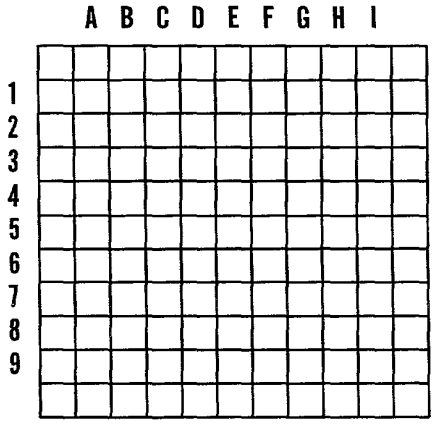


FIG. 19A

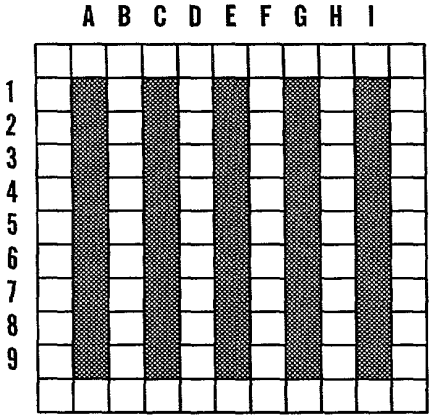


FIG. 19B

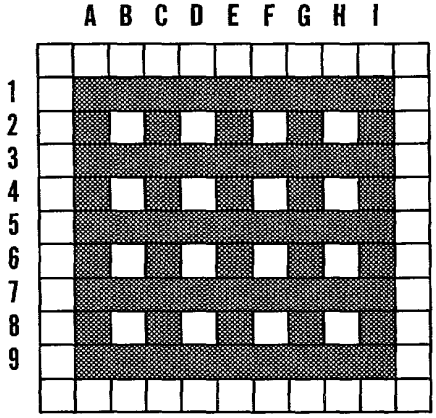


FIG. 19C

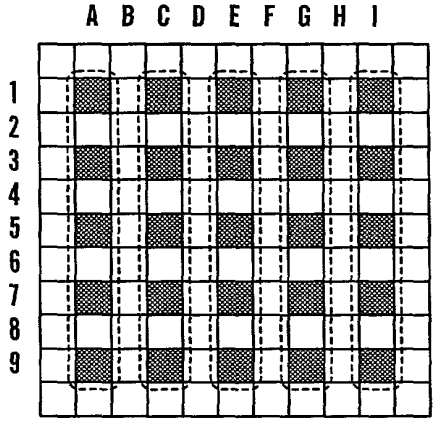


FIG. 19D

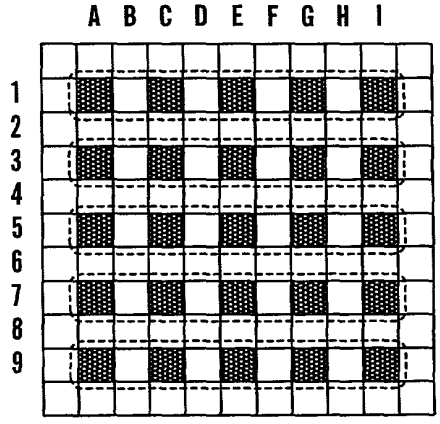


FIG. 19E

FIG. 20A

1st Tetramer additions
(columns)

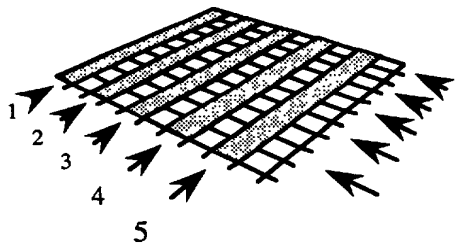
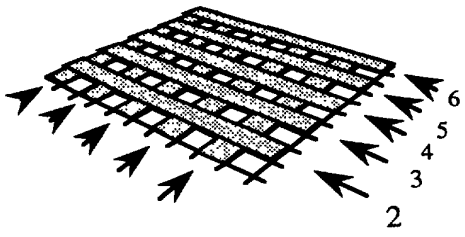


FIG. 20B

2nd Tetramer additions
(rows)



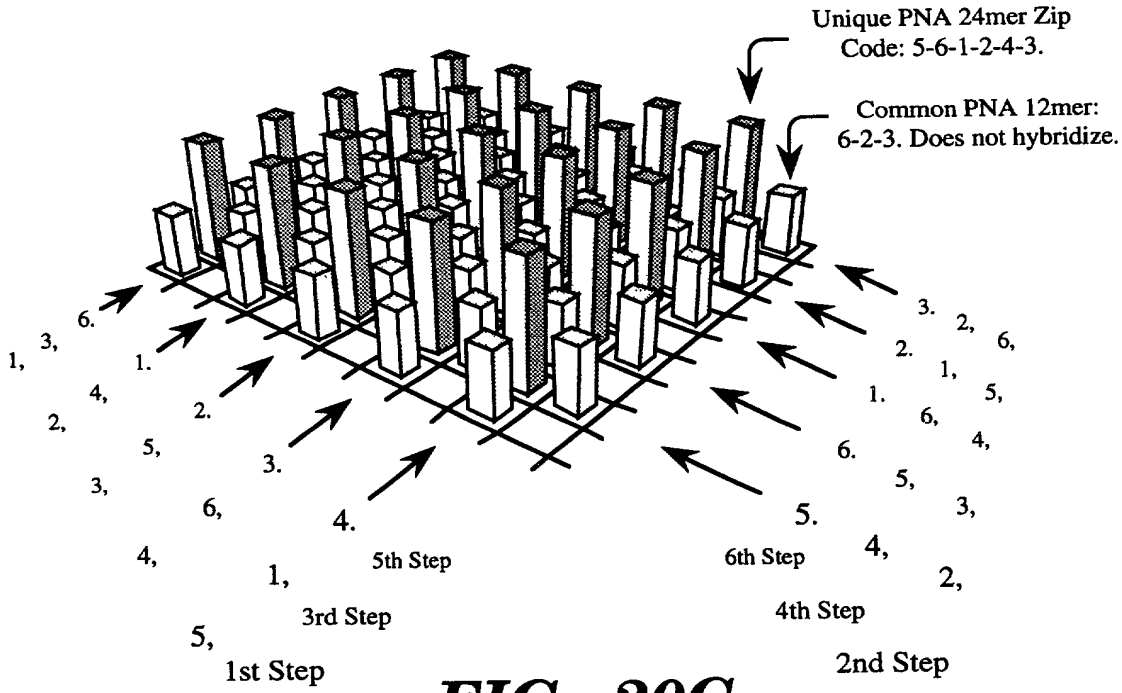


FIG. 20C

FIG. 21A

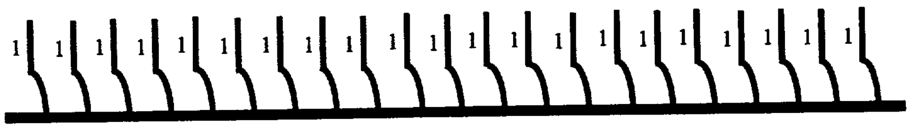

FIG. 21B


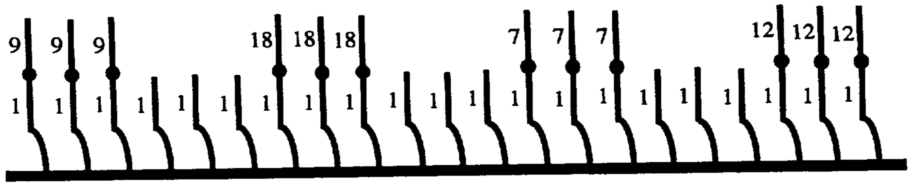
FIG. 21C


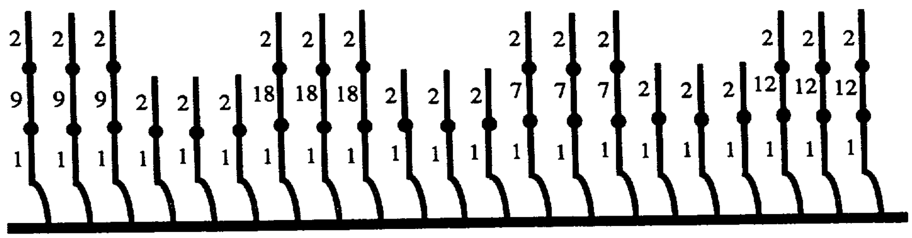
FIG. 21D


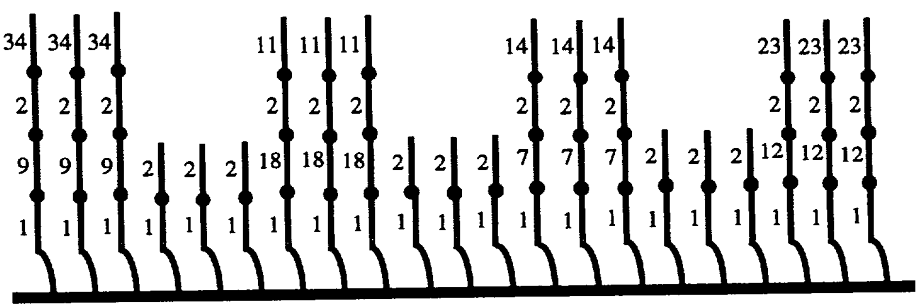
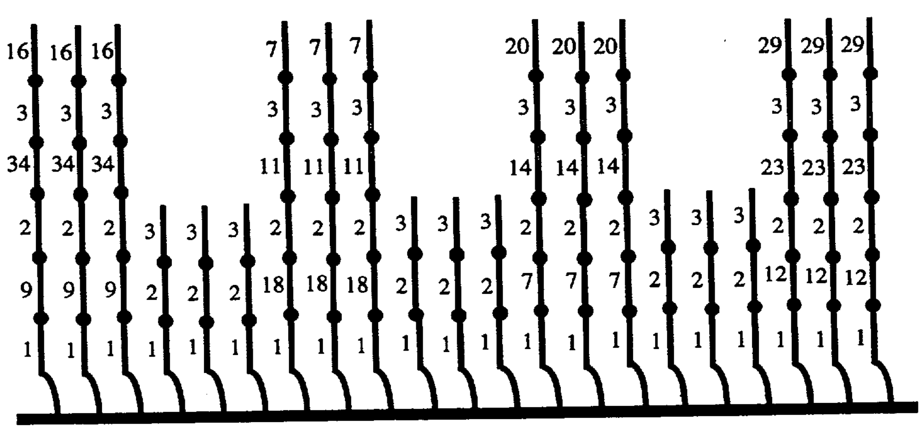
FIG. 21E


FIG. 21F


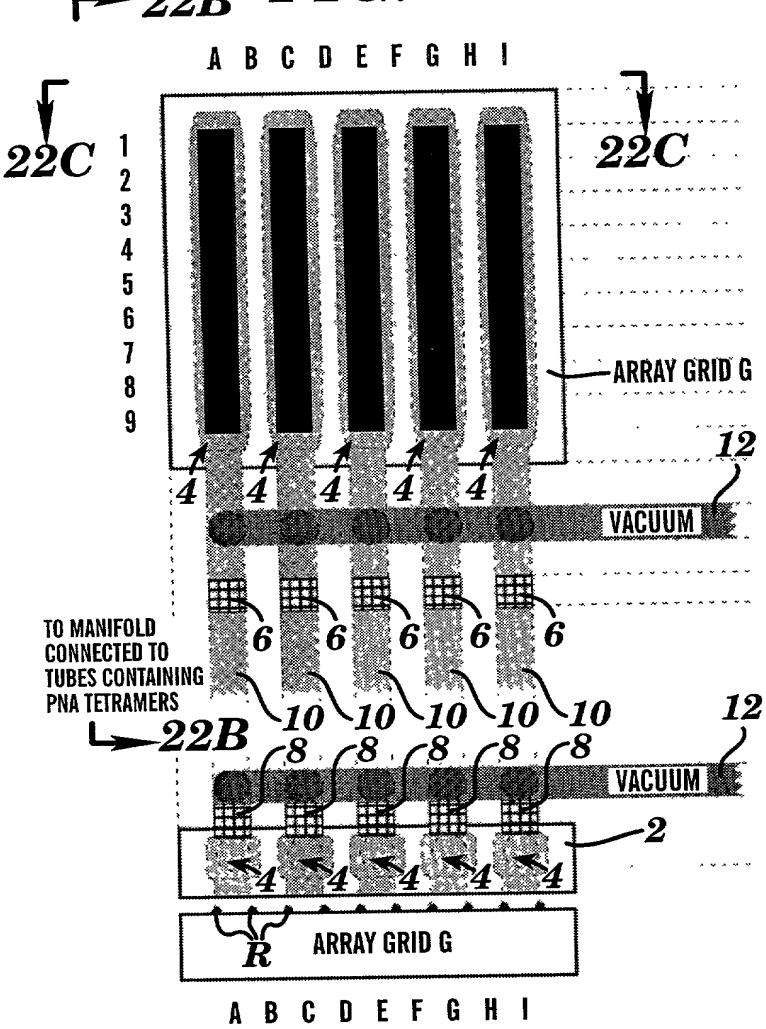


FIG. 22A

FIG. 22C

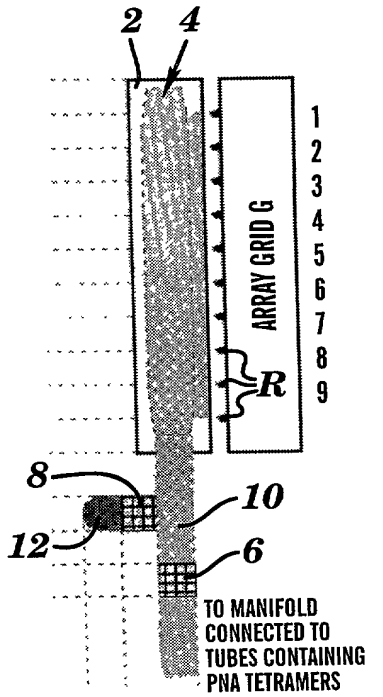


FIG. 22B

FIG. 23A

1st Tetramer additions
(columns)

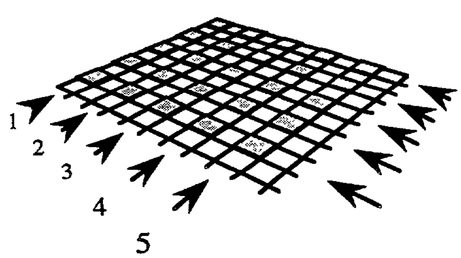
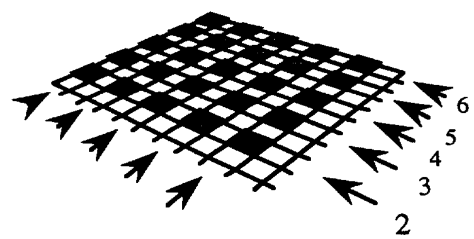


FIG. 23B

2nd Tetramer additions
(rows)



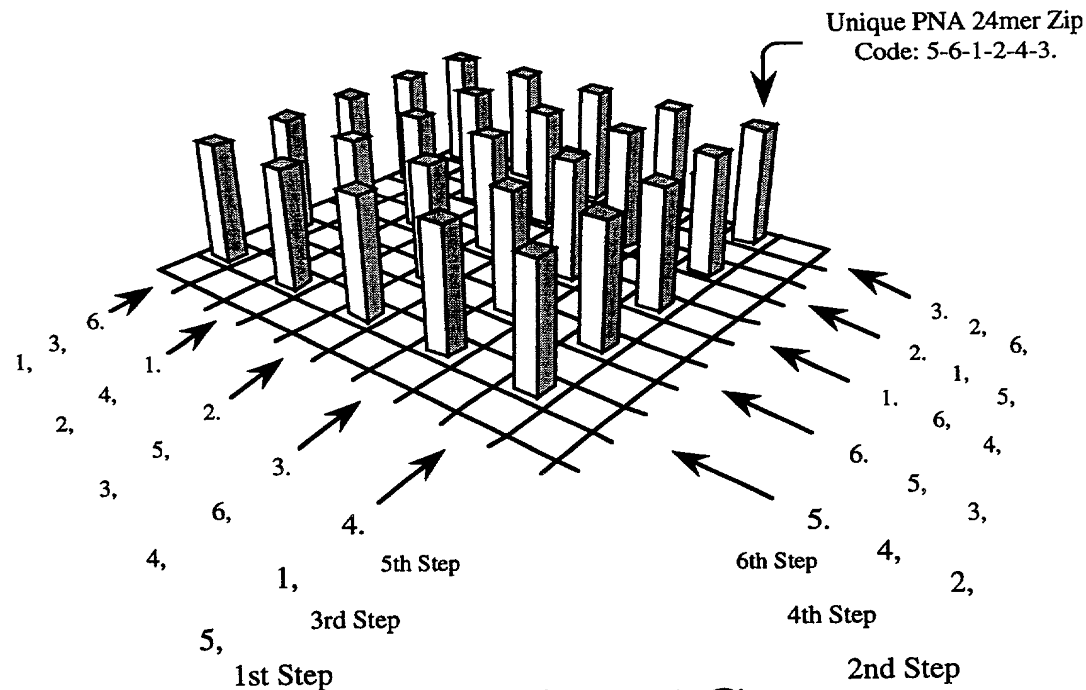


FIG. 23C

FIG. 24A

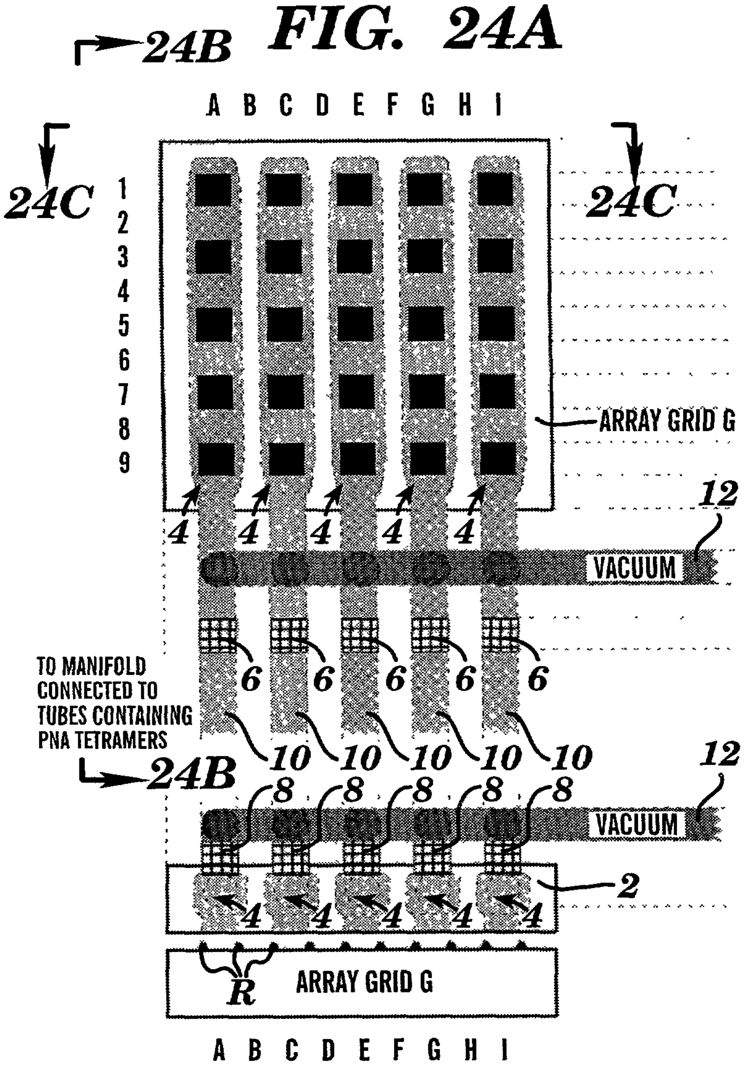


FIG. 24C

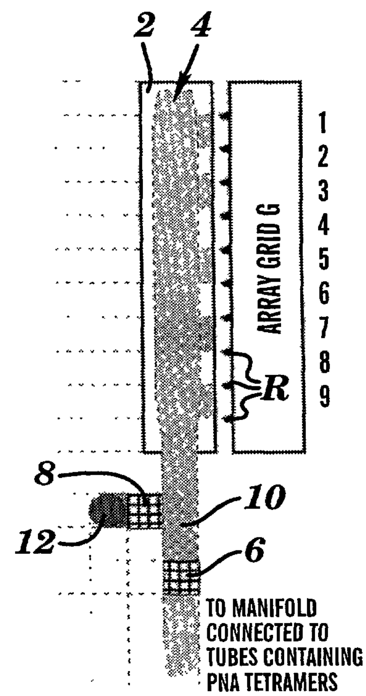


FIG. 24B

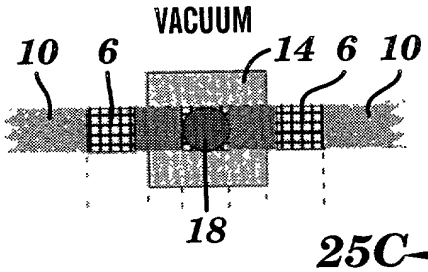


FIG. 25B

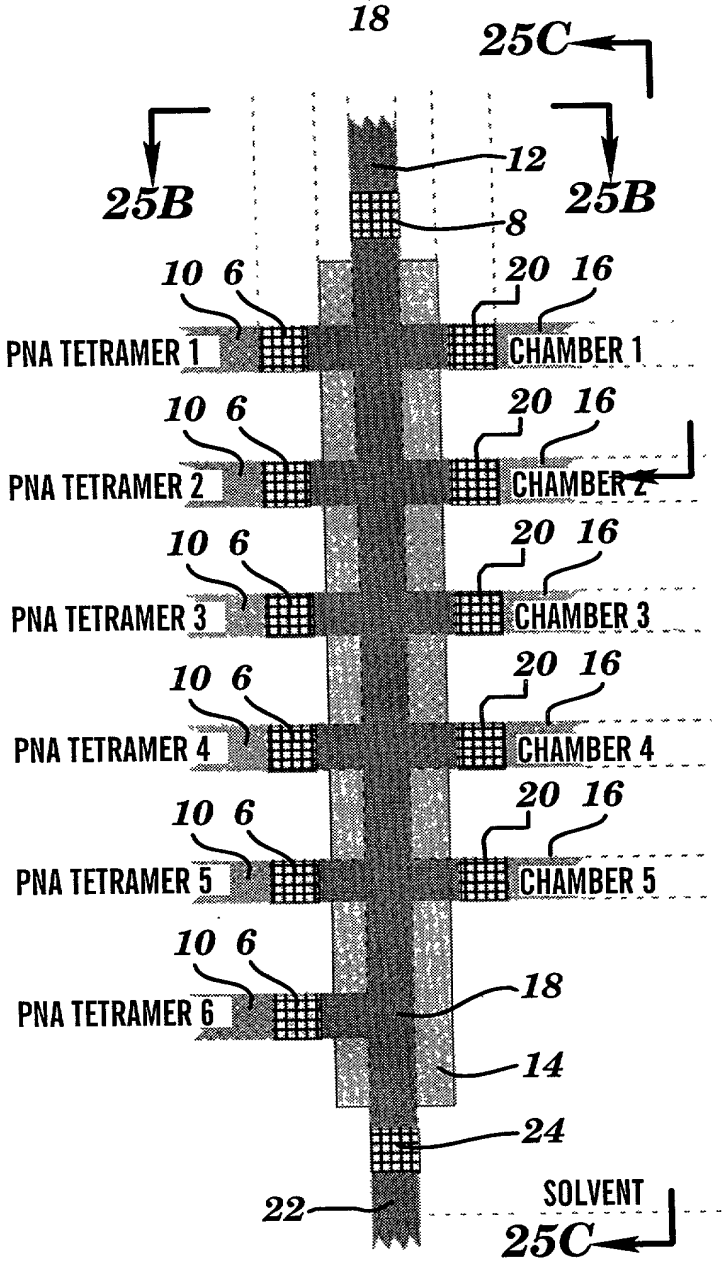


FIG. 25A

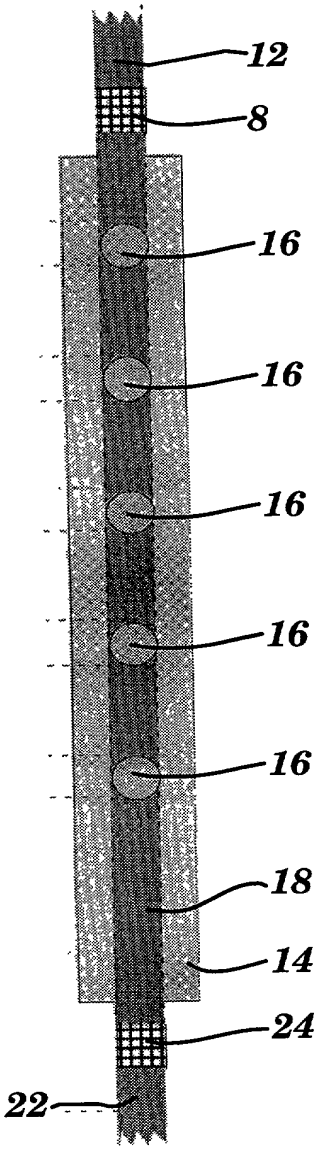


FIG. 25C

6 INPUTS AND 5 OUTPUTS

FIG. 26A

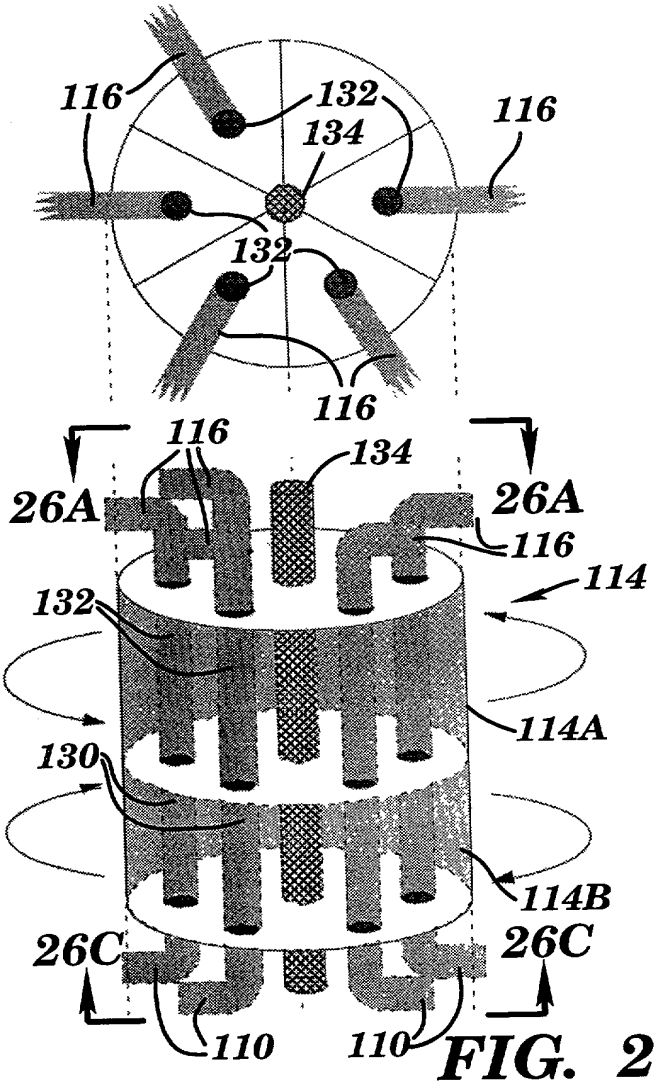


FIG. 26B

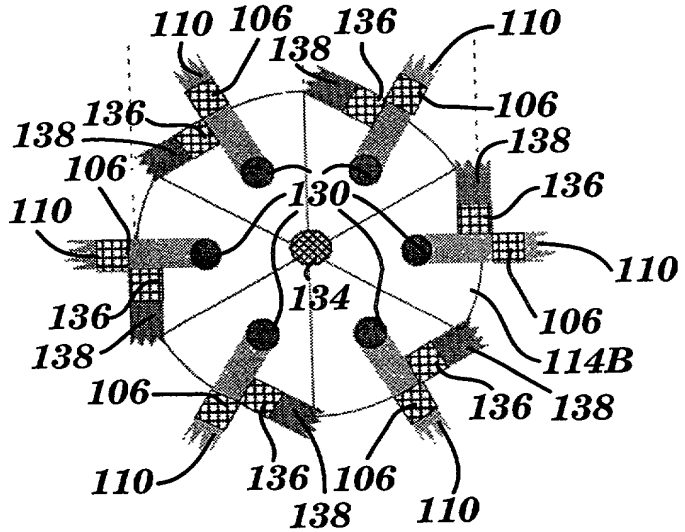


FIG. 26C

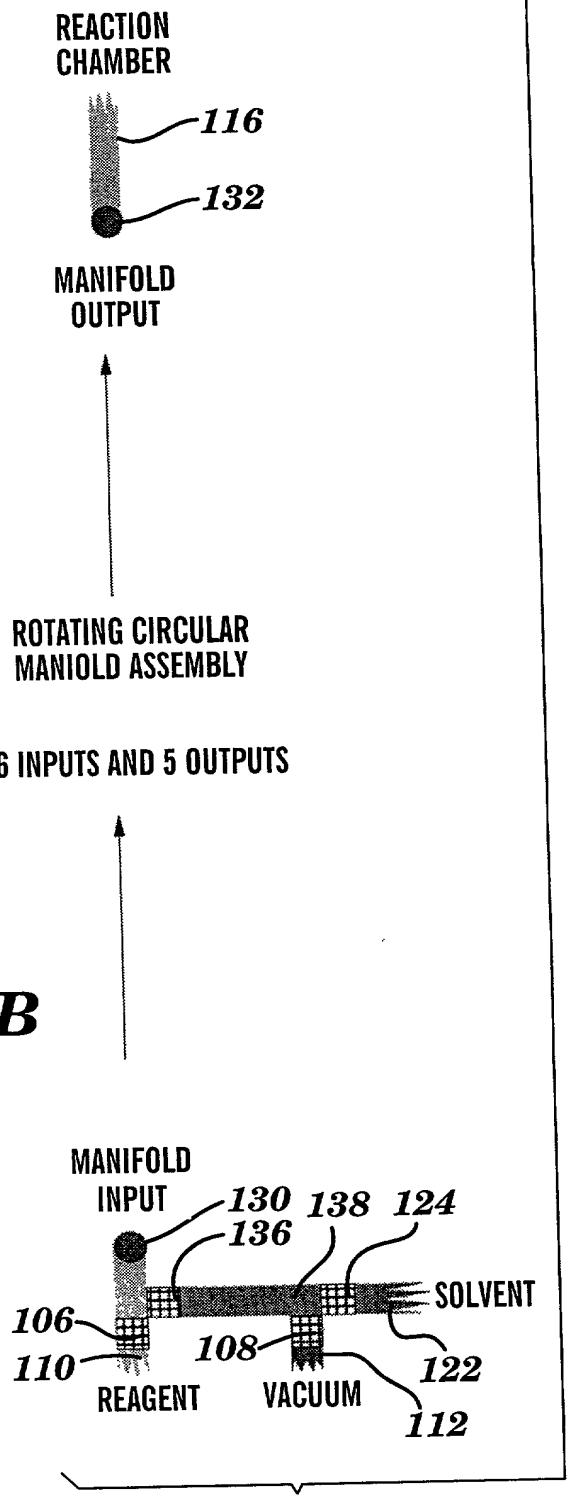


FIG. 26D

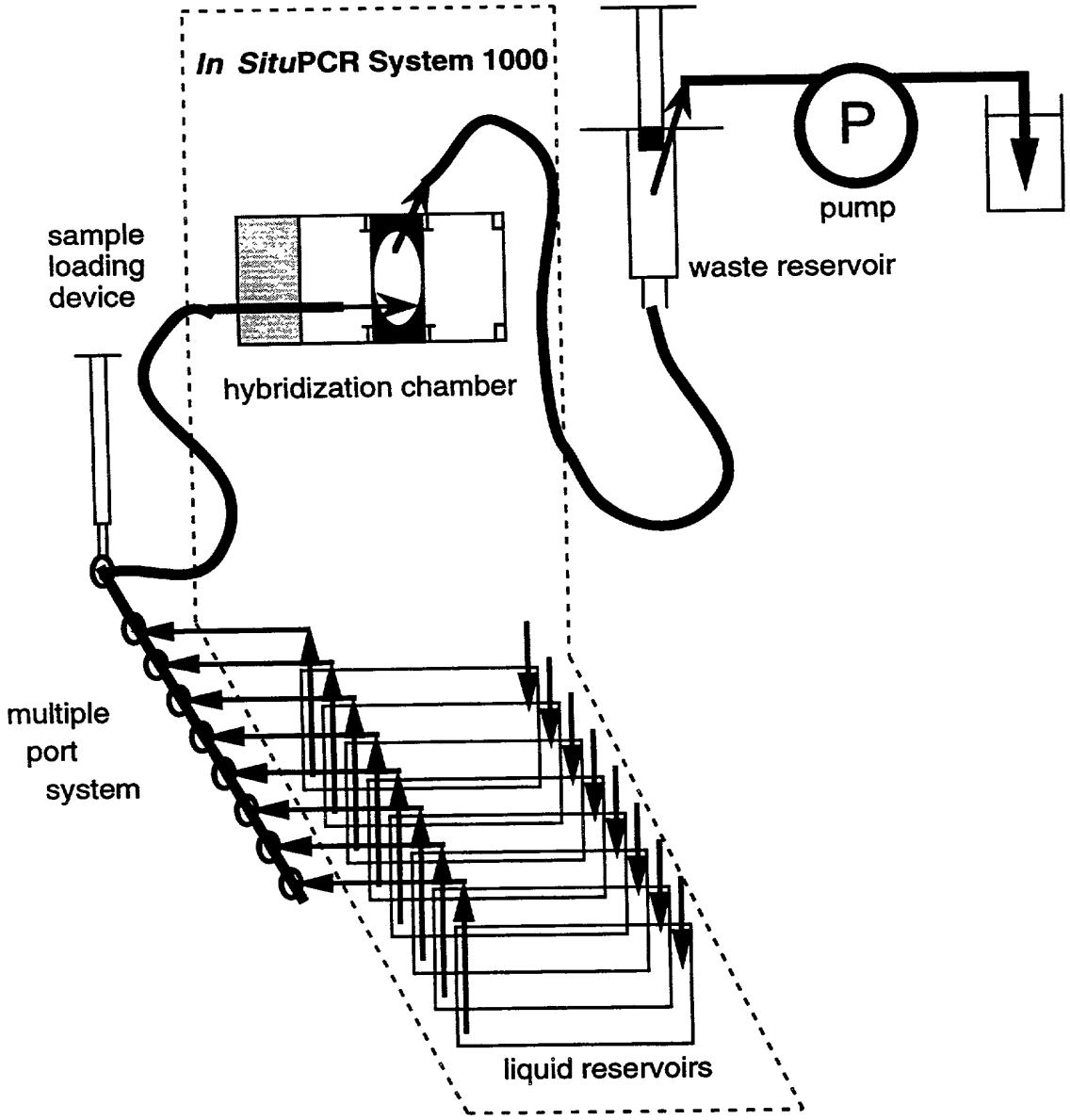

In SituPCR System 1000
P
pump
waste reservoir
sample
loading
device
hybridization chamber
multiple
port
system
liquid reservoirs

FIG. 27

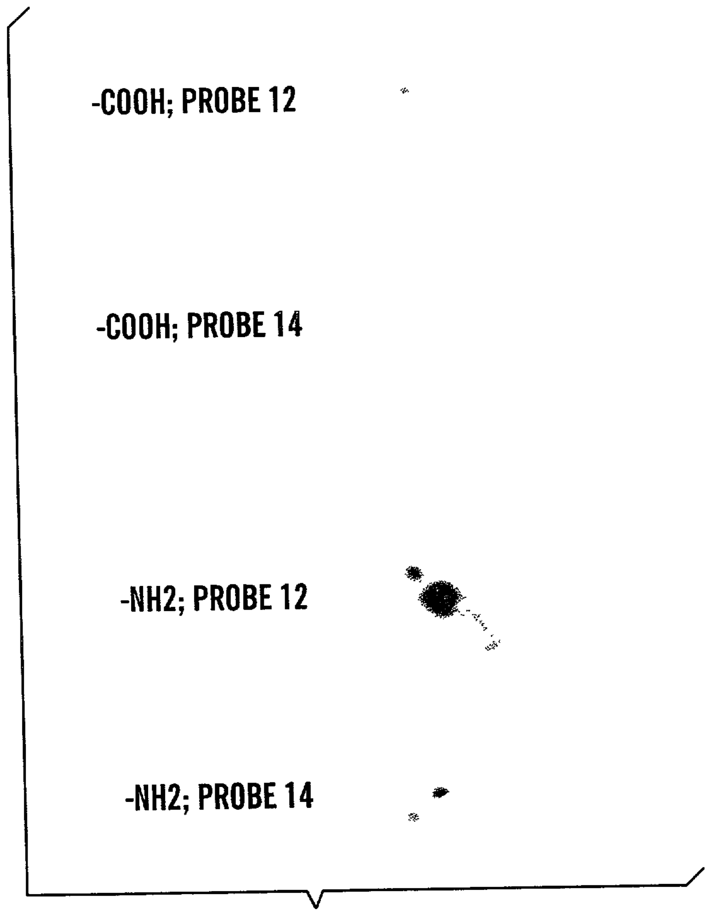


FIG. 28

2% EGDMA

2% HDDMA

4% EGDMA

FIG. 29

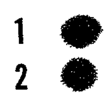
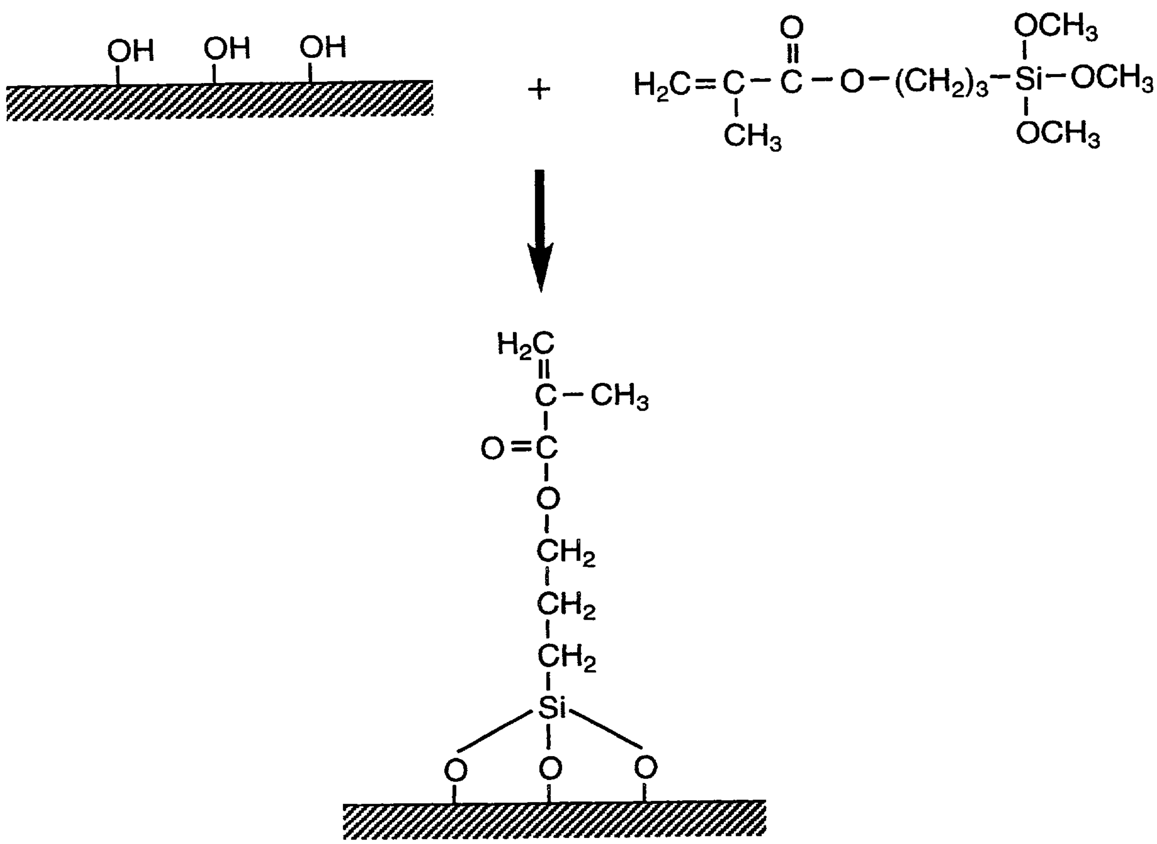


FIG. 30



OH
OH
OH
+
O
OCH3
H2C=C−C−O−(CH2)3−Si−OCH3
CH3
OCH3
H2C
C−CH3
O=C
O
CH2
CH2
CH2
Si
O
O
O

FIG. 31

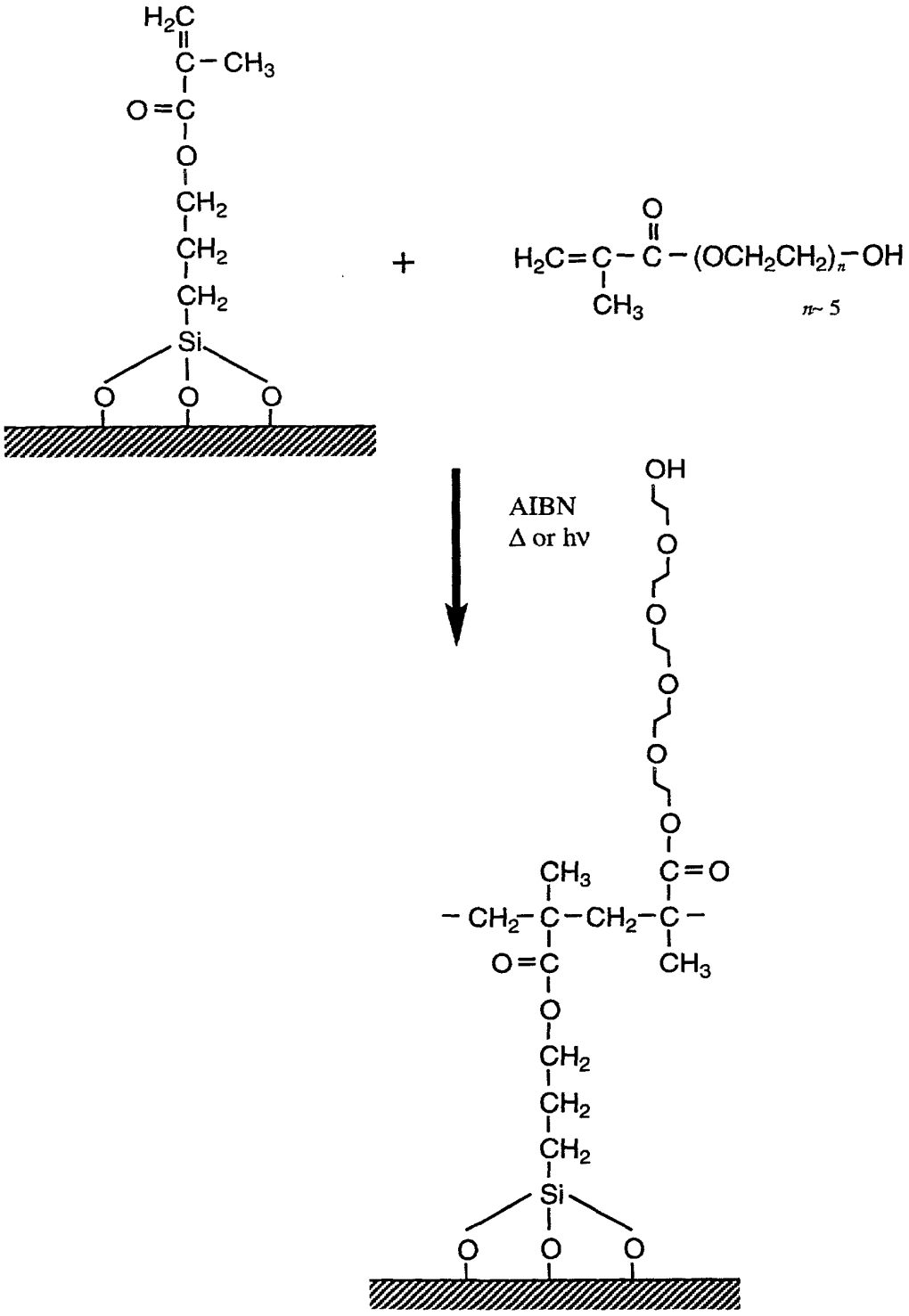


FIG. 32

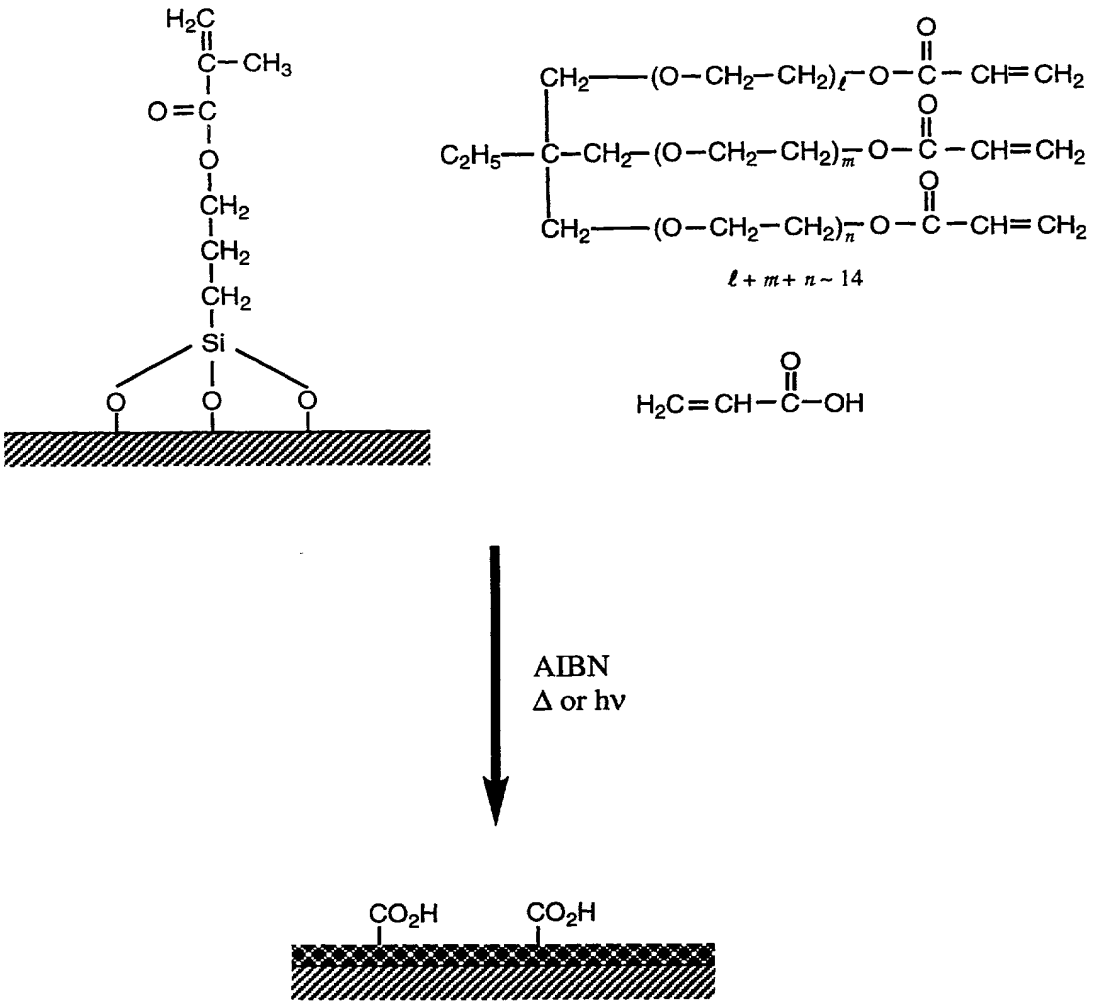


FIG. 33

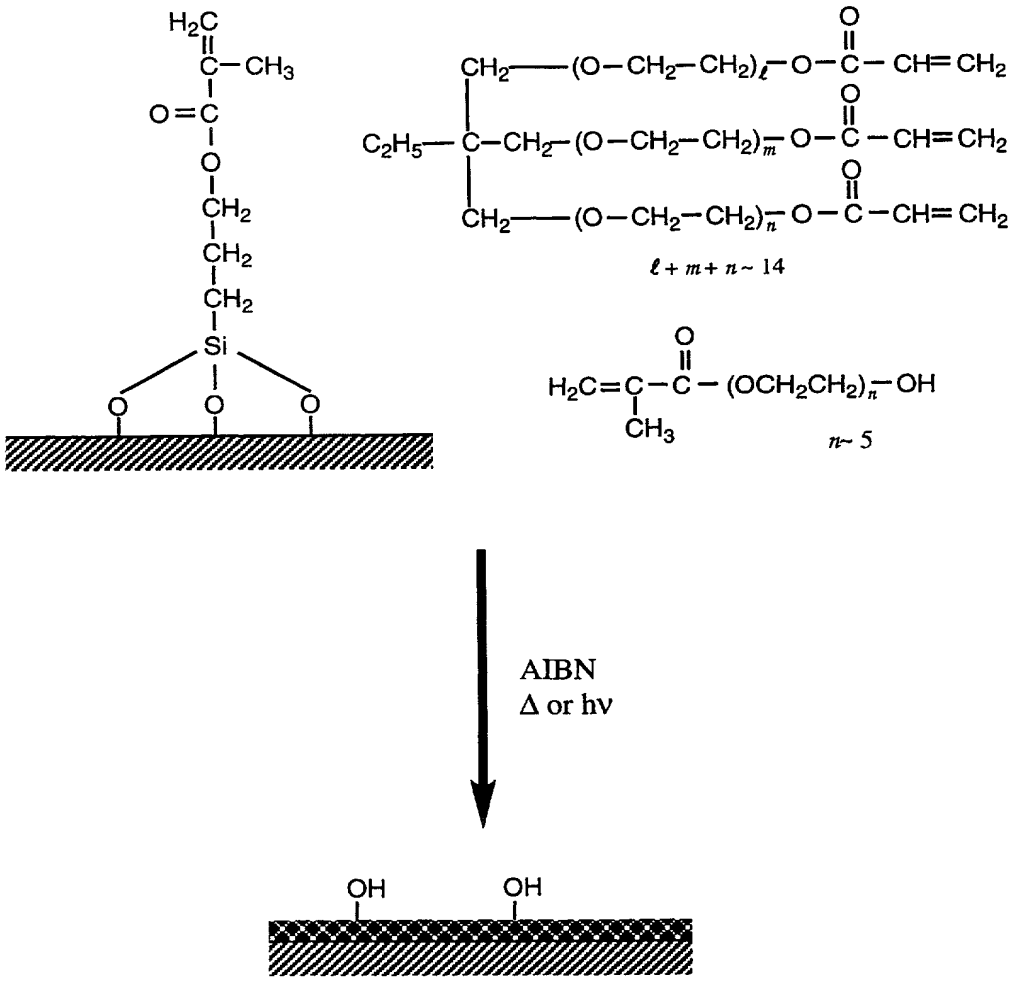

H2C
C–CH3
O=C
O
CH2
CH2
CH2
Si
O
O
O
CH2—(O–CH2–CH2)ℓ–O–C–CH=CH2
C2H5–C–CH2–(O–CH2–CH2)m–O–C–CH=CH2
CH2—(O–CH2–CH2)n–O–C–CH=CH2
ℓ + m + n ~ 14
H2C=C–C–(OCH2CH2)n–OH
CH3
n ~ 5
AIBN
Δ or hν
OH
OH

FIG. 34